

BLAST 2 SEQUENCES

This tool produces the alignment of two given sequences using **BLAST** engine for local alignment. The stand-alone executable for blasting two sequences (bl2seq) can be retrieved from [NCBI ftp site](#)

Reference: Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250

Program Matrix

Parameters used in **BLASTN** program only:

Reward for a match: Penalty for a mismatch:

☐ Use **Mega BLAST** Strand option

Open gap and extension gap penalties

gap x_dropoff expect word size Filter ☒

Sequence 1 Enter accession or GI or download from file

or sequence in FASTA format from: to:

```
qsegpavvniqaapaprtcgngsgnaetasopiaacsapryerkrivpnmpeipqeeaacgg
lnfgsgfiiskngyiltntnhvvgmgsikvllndkreylaklgsvqsdvallkidatee
lpvvkignpknlpkgewvaaigapfgfdnsvtagivsakgrslpnesytpfiqtdvainpg
nsggplfnlkgqvvginsqiysrsggfmgisfaipidvamnvaeqlkntgkvqrgqlgvii
qevsyglaqsfglkdgasgaliakilpgspaeraglgagdivlsldggeirssgdlpvmvga
itpgkevslgvwrkgeeitikaklgnaaehtgassktdeapyteqqsgtfsvesagitlqt
htdssgkhlvvrvsdaaeraglrhgdeilavrasprq
```

Sequence 2 Enter accession or GI or download from file

or sequence in FASTA format from: to:

```
mgikkvctivcivcigrlytrorvnggernavsiikakineegkpvnllrcycllqm
kvaerimachpgerfyvvlmsenrnekydyfngikdkaerayffylpyglngksfnfiptm
aelkvksmlpkkvkriylaslekvsiaaflstypdaeiktfdgtnnliressylggefav
ngaikrnfarmmvgdwsiaaktrnasdehytifkglnimddgrkrmtylplfdaselkagd
etggtrvillgspdkemkeisekaaknfniqyvaphprqtyglsgvtalnspyviedyilr
eikknphtryeytffsgaaltmkdcpnvvhvaylkapaslpedywlkpvyalfrqadipilt
fddkn
```

Comments and suggestions to blast-help@ncbi.nlm.nih.gov



Blast 2 Sequences results

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.4 [Aug-26-2002]

Matrix: **BLOSUM62** gap open: **11** gap extension: **1**
x_dropoff: **50** expect: **10.0** wordsize: **3** Filter ☒ **Align**

Sequence 1 lcl|seq_1 Length 465 091839,090 SEQ ID NO: 4

Sequence 2 lcl|seq_2 Length 371 66096,529 SEQ ID NO: 2

N significant similarity was found

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```
qsegpavvniqaapaprtqngsgnaetcsapiadsapryerfkrivpnmpeipqeeadagg  
lnfgsgfiiskngyiltnthvvgmgsikvllndkreylakligsdvqsdvallkdatee  
lpvvkignpknlpkgevwaaigapfgfdnsvtgagivsakgrslpnesytpfiqtdvainpg  
nsggplfnlkgqvvginsqiysrsggfmngisfaipidvamnvaeklntgkvqrgqlgvii  
qevsyglaqsfgldkasgaliakiilpgspaeraglgagdivlsldggeirssgdldpvmvga  
itpgkevsllgvwrkgeeitikaklgnaahtgassktdeapyteqqsgtfsvesagitlqt  
htdssgkhlvvrvsdaaeraglrhgdeilavrasprq
```

Sequence 2 Enter accession or GI or download from file

or sequence in FASTA format from: to:

```
mgik kacitvicilvicrglityctarvngernavsiikakirneegepvniicytliqm  
kvaerimachpgerfyvvlmsenrnekydyfkkqikdkaerayffhlp yglnksfnfiptm  
aelkvksmlpkvkriylaslekvsiaaflstypdaeiktfd dgtgnliqsssy lgdefsv  
ngtikrnfarmmigdwsiaktrnasdehytifkglknimddgrkrmtylplfdaselkagd  
etggtrvillgspdkemkeisekaaknfniqyvaphprqtyglsgvttlns pyviedyilr  
eikknphtreyi ytf fsgaal tmkdfpnvhvyalkpaslpedywlkpvyalftqsgipilt  
fddkn
```

Comments and suggestions to blast-help@ncbi.nlm.nih.gov



Blast 2 Sequences results

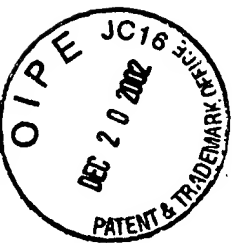
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Sequence 1 lcl|seq_1 Length 465 09/388,090 seq id no: 4

Sequence 2 lcl|seq_2 Length 371 6:096,529 seq id no: 4

No significant similarity was found

Express Mail No.: EL 500 578 282 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Jackson and Harris

RECEIVED

DEC 27 2002

Serial No.: 09/388,090

Art Unit: 1645

Filed: August 31, 1999

Examiner: S. Devi

For: NEISSERIA SPP.

Attorney Docket No.: 7969-082-999

POLYPEPTIDE, NUCLEIC ACID
SEQUENCE AND USES
THEREOF

TECH CENTER 1600/2900

Declaration of Dr. W. James Jackson Under C.F.R. § 1.132Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

I, Dr. W. James Jackson, declare and state:

1. I am a co-inventor of the above-identified application which I have read and understand.
2. The following experiments were conducted under my supervision and control. The results obtained are shown in Table 2 below. As detailed below, the results obtained clearly demonstrate that the isolated *Neisseria* polypeptide designated "NGSP" polypeptide taught in the application is useful to induce an immune response against infection by *Neisseria gonorrhea*.
3. Methods: The murine model of vaginal colonization was used to evaluate the ability of the isolated NGSP polypeptide of the invention, as described in Jerse, 1999, *Infect. Immun.* 67(11): 5699-5708, a copy of which is attached as Exhibit A, as modified as detailed below.

3a. The NGSP polypeptide (containing a His tag) was recombinantly expressed in *E. coli* JM109 containing plasmid pTLZ-NgHtr A#2 (ATCC number PTA-470) (see

Sections 8.3 and 8.4 of the application) and isolated using affinity chromatograph as described in the application in Section 5.3.

3b. Balb/c female mice, six to ten weeks of age, were immunized three times at two week intervals with isolated recombinant NGSP polypeptide. Animals were immunized, parenterally (s.c.) according to the groupings defined in Table 1 below.

3c. Via parenteral immunization, the NGSP polypeptide was coadministered with Freund's complete adjuvant (CFA) at the first immunization. An equal volume of CFA was mixed with the NGSP polypeptide antigen solution to form an emulsion. At the second and third immunizations, Freund's incomplete adjuvant (IFA) was co-administered with the NGSP polypeptide. An equal volume of IFA was mixed with the NGSP polypeptide antigen solution to form an emulsion. Animals immunized three times with PBS alone served as negative controls, while animals immunized with formalin treated *Neisseria gonorrhoea* whole cell antigen served as positive controls.

Table 1

Group Number	Antigen Amount per Dose ¹	Adjuvant
1	NGSP polypeptide (50)	CFA/IFA
2 positive control	Inactivated <i>Neisseria gonorrhoea</i> whole cell antigen	CFA/IFA
3 negative control	PBS Only	None

1 Antigen dose is given in micrograms (μ g).

3d. Retroorbital bleeds were taken prior to the first immunization and again approximately ten days following the third and final dose. Approximately ten days after the last immunization, a slow release pellet of β -estradiol (5.0mg released over 21 days; Innovative Research Inc.) was implanted under the skin of each animal to stabilize and synchronize the uterine cycle prior to and during the challenge phase of the experiment. Animals were challenged three to five days later by instilling into the vaginal canal a 20 μ l volume of PBS containing $\sim 1.0 \times 10^6$ cfu of the *N. gonorrhoea* strain MS11A.

3c. For challenge, *N. gonorrhoea* strain MS11A was grown from frozen stock cultures on Thayer Martin chocolate agar plates, collected by swabbing the sterile applicators and suspended in PBS. The *N. gonorrhoea* strain MS11A suspension was diluted with PBS to $\sim 1.0 \times 10^8$ cfu/ml using a previously generated O.D.₄₉₀ vs cfu standard curve.

3f. After challenge (48hrs post challenge) the level of *N. gonorrhoea* vaginal colonization was assessed by swabbing the vaginal canal with pediatric nasopharyngyl Dacron/Polyester swabs. Swabs were inserted gently into the canal until resistance was encountered then gently rotated (10 complete 360° turns) to collect infectious microorganisms. Swabs were removed, moistened by brief immersion into sterile PBS and used to streak plate Thayer Martin chocolate agar plates containing antibiotics (vancomycin, colistin, nystatin, trimethoprim). A standard quantitative dilution streak plating method was used to enumerate colonies. Plates were incubated at 37°C under oxygen depleted or microaerophilic conditions for 24 to 72 hours prior to counting.

Protective efficacy is expressed as percent of animals protected in a vaccination group.

4. Results: Results are presented in Table 2 below.

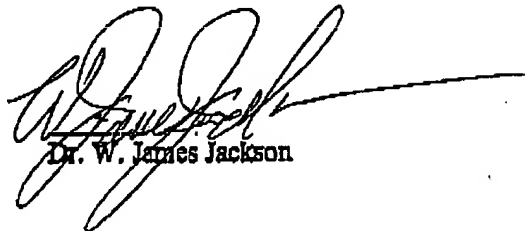
Table 2

Group Number	Ratio of Infected to Total Animals	Percent Protected
1	4/10	60%
2	3/10	70%
3	6/8	25%

5. As illustrated in Table 2, fewer mice immunized with isolated recombinant NGSP polypeptide were found to be infected with *N. gonorrhoea* after challenge compared to the unimmunized and unprotected negative controls. The level of protection conferred by NGSP polypeptide of the invention is equivalent to that obtained with the positive control, i.e., inactivated *N. gonorrhoea* whole cell antigen. These results clearly demonstrate that isolated NGSP polypeptide of the present application can confer protection against vaginal

infection by *N. gonorrhea*. These results demonstrate that the isolated NGSP polypeptide is useful to induce a protective immune response against *Neisseria gonorrhea* in a relevant animal model and thus is useful for a protective human vaccine.

I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: 12/20/02

Dr. W. James Jackson